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# Pharmacological Properties of 3-Amino-5,6,7,8-tetrahydro-2-{4-[4-(quinolin-2-yl)piperazin-1-yl]butyl}quinazolin-4(3H)-one (TZB-30878), a Novel Therapeutic Agent for Diarrhea-Predominant Irritable Bowel Syndrome (IBS) and Its Effects on an Experimental IBS Model

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## ABSTRACT

3-Amino-5,6,7,8-tetrahydro-2-{4-[4-(quinolin-2-yl)piperazin-1-yl]butyl}quinazolin-4(3H)-one (TZB-30878) is a novel compound with both 5-hydroxytryptamine (5-HT)<sub>1A</sub> agonism and 5-HT<sub>3</sub> antagonism effects. We hypothesized that TZB-30878 might have benefits from these dual effects as a medication for diarrhea-predominant irritable bowel syndrome (d-IBS), and these studies were designed to confirm the pharmacological properties of TZB-30878 and its efficacy in an IBS-like animal model. The binding assays demonstrated that [<sup>3</sup>H]TZB-30878 selectively binds to human 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors, with *K<sub>d</sub>* values of  $0.68 \pm 0.03$  and  $8.90 \pm 1.73$  nM, respectively. Systemic administration of TZB-30878 inhibited 5-HT-induced bradycardia in a dose-dependent manner in rats. In behavioral assays TZB-30878 produced signs of 5-HT syndrome in rats. These results suggest that TZB-30878 has dual effects as a 5-HT<sub>1A</sub> receptor agonist and a 5-HT<sub>3</sub> receptor antagonist. Fi-

nally, we evaluated the effects of TZB-30878 on wrap restraint stress-induced defecation in an IBS-like model in rats. TZB-30878 (1–10 mg/kg p.o.) normalized stress-induced defecation in a dose-dependent manner, whereas the 5-HT<sub>1A</sub> agonist tandospirone (30 and 100 mg/kg p.o.) and the 5-HT<sub>3</sub> antagonist alosetron (1–10 mg/kg p.o.) did not show such effects. Furthermore, this efficacy of TZB-30878 was partly antagonized by a 5-HT<sub>1A</sub> antagonist, [O-methyl-3H]-N-(2-{4-(2-methoxyphenyl)-1-piperazinyl}ethyl)-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635). These results suggest that 5-HT<sub>1A</sub> receptor agonism and 5-HT<sub>3</sub> receptor antagonism contribute to the efficacy of TZB-30878 in the IBS-like model. The efficacy of TZB-30878 supports the concept that the presence of both actions, namely 5-HT<sub>1A</sub> receptor agonism and 5-HT<sub>3</sub> receptor antagonism, could be an important mechanism in the treatment of d-IBS.

Irritable bowel syndrome (IBS) is one of the most common functional disorders of the gastrointestinal tract, with major symptoms such as abdominal discomfort or pain accompanied by constipation or diarrhea (Talley et al., 1991; Drossman et al., 1993). The syndrome has been considered to be associated with psychological stress, abnormal gut motility,

and abdominal visceral hyperalgesia (Whitehead and Crowell, 1991; Camilleri and Choi, 1997). Indeed, anxiolytics and ant motility agents have been used for the treatment of this disorder (Friedman, 1991; Talley et al., 1991). However, the existing medications have not been adequately effective for patients with IBS.

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter found in both the brain and gut and is probably involved in brain-gut interactions in normal volunteers and possibly to a greater degree in patients with IBS (Kim and

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**ABBREVIATIONS:** IBS, irritable bowel syndrome; 5-HT, 5-hydroxytryptamine, serotonin; d-IBS, diarrhea-predominant irritable bowel syndrome; TZB-30878, 3-amino-5,6,7,8-tetrahydro-2-{4-[4-(quinolin-2-yl)piperazin-1-yl]butyl}quinazolin-4(3H)-one; GTP- $\gamma$ S, guanosine 5'-[ $\gamma$ -thio]triphosphate; tandospirone, (1R,2S,3R,4S)-N-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,3-bicyclo[2.2.1]heptanedicarboximide dihydrogen; alosetron, 2,3,4,5-tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)methyl]-1H-pyrido[4,3-b]indol-1-one; 8-OH-DPAT, 2-(N,N-di-n-propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene; WAY-100635, [O-methyl-3H]-N-(2-{4-(2-methoxyphenyl)-1-piperazinyl}ethyl)-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; CHO, Chinese hamster ovary; BRL-43694, granisetron; HEK, human embryonic kidney; BJ, Bezold-Jarisch; CNS, central nervous system; 1-PP, 1-(2-pyrimidinyl)piperazine.

Camilleri, 2000; Talley, 2001). Azapirones, such as buspirone and tandospirone, have been used as a class of anxiolytics that are less sedative and more anxiolytic than the benzodiazepines (Robinson, 1991; Sasa, 1997). The mechanism of their anxiolytic action is considered to be an agonistic action for one of the 5-HT receptor subtypes, the 5-HT<sub>1A</sub> receptors (Traber and Glaser, 1987; Sasa, 1997). In clinical trials, the improvement rating with tandospirone was higher than that with the placebo in patients with IBS (Kimura et al., 1992). This indicates the possible involvement of the 5-HT<sub>1A</sub> receptors in the treatment of IBS.

Clinical studies demonstrated accelerated transit through the small bowel or colon in some patients with diarrhea-predominant IBS (d-IBS) (Cann et al., 1983; Vassallo et al., 1992). It has been suggested that gastrointestinal transit may mediate the development of symptoms in IBS. Previous studies have focused on the role of the serotonin system in intestinal motor function, particularly with the 5-HT<sub>3</sub> receptors. A selective 5-HT<sub>3</sub> receptor antagonist, ondansetron, has been shown to delay colonic transit in healthy volunteers (Gore et al., 1990; Talley et al., 1990). Another 5-HT<sub>3</sub> receptor antagonist, granisetron, has been shown to reduce postprandial rectal motor activity in IBS patients (Prior and Read, 1993). In addition, recent studies have demonstrated the effectiveness of a 5-HT<sub>3</sub> receptor antagonist, alosetron, in women with diarrhea-predominant IBS (Camilleri et al., 2000, 2001; Lembo et al., 2001). Thus, 5-HT<sub>3</sub> receptors are thought to be related to the pathophysiology of IBS.

We considered that the development of a unique compound to stabilize mental stress and control gastrointestinal motility could be successful in the treatment of IBS. We have focused on 5-HT<sub>1A</sub> receptor agonism and 5-HT<sub>3</sub> receptor antagonism as appropriate activity for a medication for patients with d-IBS and have synthesized a novel compound, TZB-30878, which has the dual pharmacological activities of 5-HT<sub>1A</sub> receptor agonism and 5-HT<sub>3</sub> receptor antagonism. We believe that having these dual actions could be valuable for the treatment of IBS. TZB-30878 is rapidly absorbed after oral administration, permeates the blood-brain barrier, and is eliminated via hepatic metabolism.

In the present study, we evaluated the following pharmacological profiles of TZB-30878. 1) The binding affinities for human recombinant 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors were determined using [<sup>3</sup>H]TZB-30878; 2) the functional activities in vitro were determined in the GTPγS binding assay for 5-HT<sub>1A</sub> receptors and in the contraction-response assay for 5-HT<sub>3</sub> receptors; 3) 5-HT<sub>1A</sub> agonistic action in vivo was evaluated based on the 5-HT behavioral syndrome in rats; and 4) 5-HT<sub>3</sub> antagonistic action in vivo was evaluated based on 5-HT-induced bradycardia (von Bezold-Jarisch reflex) in anesthetized rats. The main aim of this study was to evaluate the effects of TZB-30878 on wrap restraint stress-induced defecation in rats, which is believed to be a pathological model for IBS (Williams et al., 1988). More specifically, we examined the value of having both actions for the treatment of IBS.

## Materials and Methods

**Animals.** Male Sprague-Dawley rats (Charles River Laboratories Japan, Inc., Yokohama, Japan), weighing 170 to 230 g, and female Hartley guinea pigs (Japan SLC, Inc., Shizuoka, Japan), weighing

280 to 350 g, were used. Animals were maintained under controlled environmental conditions with constant temperature (22°C) and a 12-h light/dark cycle (lighted from 8:00 AM to 8:00 PM) and were allowed free access to feed and water. All studies were conducted after approval by the Animal Research Committee of ASKA Pharmaceutical Co., Ltd., based on the criteria described in the Rules for the Care and Use of Laboratory Animals.

**Drugs.** All drugs were prepared fresh at each use. The drugs were administered at a dose volume of 2 ml/kg (i.p., s.c., or intraduodenal) or 5 ml/kg (p.o.). TZB-30878, tandospirone, and alosetron were prepared by ASKA Pharmaceutical Co., Ltd. (Kawasaki, Japan). [<sup>3</sup>H]TZB-30878, 2-(N,N-di(2,3-(n)-<sup>3</sup>H)propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene (8-OH-[<sup>3</sup>H]DPAT), and GTP[γ-<sup>35</sup>S] were obtained from Amersham Biosciences (Buckinghamshire, England). [<sup>3</sup>H]BRL-43694 (granisetron) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA). 5-HT creatinine sulfate complex, 2-methyl-5-HT maleate salt, 8-OH-DPAT, and WAY-100635 maleate salt were obtained from Sigma-Aldrich (St. Louis, MO). Ethyl carbamate (urethane) was obtained from Tokyo Chemical Industry (Tokyo, Japan).

**Radioligand Binding Assays.** Human 5-HT<sub>1A</sub> receptor (cloned human serotonin receptor subtype 1A produced in CHO cells; PerkinElmer Life and Analytical Sciences) was thawed on ice and diluted with buffer A (50 mM Tris-HCl, 10 mM MgSO<sub>4</sub>, 0.5 mM EDTA, and 0.1% (w/v) ascorbic acid, pH 7.4) to prepare a human 5-HT<sub>1A</sub> receptor membrane preparation. The following procedures were performed in the three experiments. Twenty microliters of buffer A, 20 μl of [<sup>3</sup>H]TZB-30878 solutions (final concentrations: 0.01–10 nM), and 500 μl of the human 5-HT<sub>1A</sub> receptor membrane preparation (including 1 U) were placed in tubes and mixed to prepare reaction mixtures in triplicate (nonspecific binding was determined in the presence of 10 μM TZB-30878). The reaction mixtures were incubated at 27°C for 1 h. The incubation was terminated by filtering the reaction mixtures through GF/C filters soaked in 0.3% polyethylenimine and 50 mM Tris-HCl (pH 7.4) using a Brandel cell harvester (Brandel Inc., Gaithersburg, MD). The filters were washed twice with 5 ml of cold buffer C (50 mM Tris-HCl, pH 7.4). Radioactivity retained on the filters was counted with a liquid scintillation counter (LSC-5100; Aloka Co., Ltd., Tokyo, Japan). The assay procedure for the competitive analysis was essentially the same as described above. Assays were carried out after saturation binding of 8-OH-[<sup>3</sup>H]DPAT (final concentrations: 0.01–10 nM) to obtain the binding affinity (K<sub>d</sub> value) of 8-OH-[<sup>3</sup>H]DPAT. Twenty microliters of buffer A or the test drugs (final concentrations: 0.03–1000 nM), 20 μl of 8-OH-[<sup>3</sup>H]DPAT solution (the final concentration was close to the K<sub>d</sub> value), and 500 μl of the human 5-HT<sub>1A</sub> receptor membrane preparation (including 1 U) were placed in tubes and mixed to prepare reaction mixtures in triplicate. Nonspecific binding was determined in the presence of 10 μM 8-OH-DPAT.

**Human 5-HT<sub>3</sub> receptor (human 5HT<sub>3</sub> serotonin receptor, a membrane preparation of HEK293 cells expressing human 5-HT<sub>3</sub> receptor genes; PerkinElmer Life and Analytical Sciences) was thawed on ice and diluted with buffer B (50 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, and 1 mM EDTA, pH 7.5) to prepare the human 5-HT<sub>3</sub> receptor membrane preparation. The following procedures were performed in the three experiments. Twenty microliters of buffer B, 20 μl of [<sup>3</sup>H]TZB-30878 solutions (final concentrations: 0.03–20 nM), and 500 μl of the human 5-HT<sub>3</sub> receptor membrane preparation (including 1 U) were placed in tubes and mixed to prepare reaction mixtures in triplicate (nonspecific binding was determined in the presence of 10 μM TZB-30878). The reaction mixtures were incubated at 25°C for 1 h. The incubation was terminated by filtering the reaction mixtures through GF/B filters soaked in 0.5% polyethylenimine and 50 mM Tris-HCl (pH 7.4) using a Brandel cell harvester. The filters were washed twice with 5 ml of cold buffer C (50 mM Tris-HCl, pH 7.4). Radioactivity retained on the filters was counted with a liquid scintillation counter. The assay procedure for the competitive analysis was essentially the same as that described above. Assays were carried out after saturation binding of [<sup>3</sup>H]BRL-43694 (final concentra-**

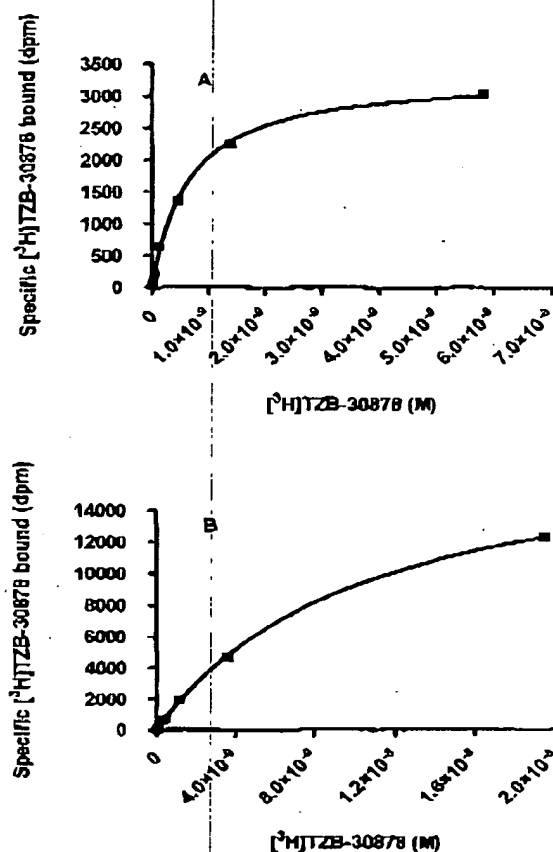


Fig. 1. Saturation binding curve of  $[^3\text{H}]\text{TZB-30878}$  to human  $5\text{-HT}_{1A}$  receptors expressed in CHO cells (A) and  $5\text{-HT}_2$  receptors expressed in HEK293 cells (B). Each receptor membrane preparation was incubated with increasing concentrations of  $[^3\text{H}]\text{TZB-30878}$  (0.01–10 nM  $5\text{-HT}_{1A}$  or 20 nM  $5\text{-HT}_2$ ). Nonspecific binding was detected in the presence of  $10 \mu\text{M}$  TZB-30878. The figure shows one of three analyses.

tions: 0.01–10 nM) to obtain the  $K_d$  value of  $[^3\text{H}]\text{BRL-43694}$ . Twenty microliters of buffer B or the test drugs (final concentrations: 0.03–1000 nM), 20  $\mu\text{l}$  of  $[^3\text{H}]\text{BRL-43694}$  solution (the final concentration was close to the  $K_d$  value), and 500  $\mu\text{l}$  of the human  $5\text{-HT}_2$  receptor membrane preparation (including 1 U) were placed in tubes and mixed to prepare reaction mixtures in triplicate. Nonspecific binding was determined in the presence of  $10 \mu\text{M}$  granisetron. In addition, TZB-30878 was tested in more than 20 other receptor and transporter assays (Cerap, Celle l'Évêque, France).

**$5\text{-HT}_{1A}$  Agonist-Induced  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  Binding Assays.** Assays were carried out using the method described previously (Stanton and Beer, 1997) with partial modification. Human  $5\text{-HT}_{1A}$  receptor (cloned human serotonin receptor subtype 1A produced in CHO cells; PerkinElmer Life and Analytical Science) was thawed on ice and diluted with incubation buffer (20 mM HEPES, 3 mM  $\text{MgCl}_2$ , and 120 mM NaCl, pH 7.4). Membranes were incubated with GDP (20  $\mu\text{M}$ ) and the test drugs for 20 min at  $30^\circ\text{C}$  in a volume of 900  $\mu\text{l}$  and then placed on ice for 15 min.  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  (100 pM) was added to all tubes to yield a final volume of 1 ml, and the tubes were further incubated for 30 min at  $30^\circ\text{C}$ . Incubation was terminated by filtering the mixtures through GF/B filters using a Brandel cell harvester. The filters were washed twice with 5 ml of cold wash buffer (20 mM HEPES and 3 mM  $\text{MgCl}_2$ , pH 7.4 at  $4^\circ\text{C}$ ). Radioactivity retained on the filters was counted by a liquid scintillation counter. Nonspecific binding was determined in the presence of  $10 \mu\text{M}$  GTP- $\gamma\text{S}$ .

**Inhibition of  $5\text{-HT}_2$  Receptor-Mediated Contraction in Guinea Pig Ileum.** Hartley guinea pigs were sacrificed, and their

# Dual Effects of TZB-30878 on IBS Model

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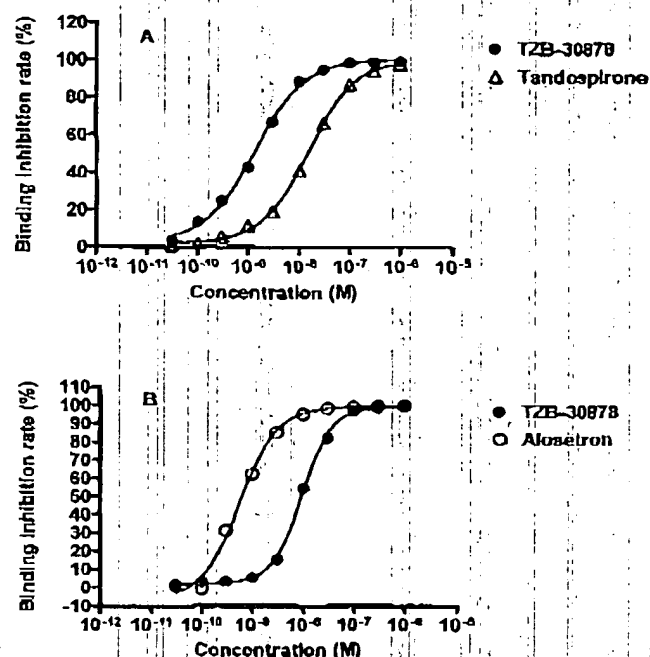


Fig. 2. Substitution curve of 8-OH- $[^3\text{H}]\text{DPAT}$  by TZB-30878 and tandospirone to human  $5\text{-HT}_{1A}$  receptors expressed in CHO cells (A) or  $[^3\text{H}]\text{BRL-43694}$  by TZB-30878 and alosetron to  $5\text{-HT}_2$  receptors expressed in HEK293 (B). Each receptor membrane preparation was incubated with various concentrations of the drugs. Nonspecific binding was detected in the presence of  $10 \mu\text{M}$  8-OH-DPAT ( $5\text{-HT}_{1A}$ ) or granisetron ( $5\text{-HT}_2$ ). The figure shows one of three analyses.

ileums were isolated. Longitudinal muscle strips including the myenteric plexus were prepared from a 1-cm-long segment of the ileum (Butler et al., 1990). The ileum preparations were suspended in an organ bath containing Tyrode's solution [137 mM NaCl, 3 mM KCl, 2 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 12 mM  $\text{NaHCO}_3$ , 0.4 mM  $\text{NaH}_2\text{PO}_4$ , and 6 mM D-(+)-glucose], warmed to  $37^\circ\text{C}$  and aerated with a mixture of 5%  $\text{CO}_2$  and 95%  $\text{O}_2$ . Isotonic contractions under a loading tension of 1 g were recorded using an isotonic force transducer (TD-112S; Nihon Kohden, Tokyo, Japan). Experiments were started after stable contractions induced by  $10 \mu\text{M}$  2-methyl-5-HT were obtained at least three times. The vehicle (dimethyl sulfoxide) or the test drugs were added to the organ bath, and the preparations were exposed to the vehicle or test drugs for 20 min. Then 2-methyl-5-HT ( $10 \mu\text{M}$ ) was added to the organ bath, and the contractions were recorded.

**$5\text{-HT}_{1A}$  Receptor-Mediated Behavior and Hypothermia in Rats.** Rats were acclimated to the test environment for 2 weeks before testing and conditioned to the test procedures during this period. On the day of the experiment, rats were acclimated to the test cage for 1 h. TZB-30878 or vehicle (saline containing diluted hydrochloric acid) was injected i.p., and then  $5\text{-HT}_{1A}$  receptor-mediated behavior (flat body posture and lower lip retraction) was measured in the test cage. Behavioral responses were measured at 5, 10, 20, and 30 min after administration using a 0 to 3 scale as described previously in the literature (Foreman et al., 1993). The maximum score obtained from each animal during the observation period was designated as the individual score. The rectal temperature was recorded before and at 30 min after administration of the test drugs using a thermistoprobe (MGAM-219; Nihon Kohden) that was inserted into the rectum, 3 cm from the anal orifice. The difference between the temperatures measured before and after administration was designated as the index of hypothermia.

**$5\text{-HT}$ -Induced Bradycardia (von Bezold-Jarisch Reflex) in Rats.** The surgical procedures and mean heart rate recordings were performed as follows. Animals were anesthetized with urethane at a

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TABLE 1

IC<sub>50</sub> values of TZB-30878 to various receptors and transportersAll experiments were demonstrated in Cerep except for 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> (in ASKA; pharmaceutical Co., Ltd.).

Receptor or Transporter	Subtype	Membrane Source	Ligand	IC <sub>50</sub>
				M
Adenosine	A <sub>1</sub>	Human recombinant	[ <sup>3</sup> H]DPCPX	>1 × 10 <sup>-8</sup>
	A <sub>2A</sub>	Human recombinant	[ <sup>3</sup> H]CGS21680	>1 × 10 <sup>-6</sup>
	A <sub>2B</sub>	Human recombinant	[ <sup>125</sup> I]AB-MECA	>1 × 10 <sup>-6</sup>
Adrenaline	α <sub>1</sub>	Rat cerebral cortex	[ <sup>3</sup> H]Prazosin	>1 × 10 <sup>-6</sup>
	α <sub>2</sub>	Rat cerebral cortex	[ <sup>3</sup> H]RX821002	>1 × 10 <sup>-6</sup>
	β <sub>1</sub>	Human recombinant	[ <sup>3</sup> H](+)-CGP12177	>1 × 10 <sup>-6</sup>
Dopamine	D <sub>1</sub>	Human recombinant	[ <sup>3</sup> H]SCH23390	>1 × 10 <sup>-6</sup>
	D <sub>2</sub>	Human recombinant	[ <sup>3</sup> H]Spiperone	>1 × 10 <sup>-6</sup>
GABA	Nonselective	Rat cerebral cortex	[ <sup>3</sup> H]GABA	>1 × 10 <sup>-6</sup>
Histamine	H <sub>1</sub>	Human recombinant	[ <sup>3</sup> H]Pyramine	>1 × 10 <sup>-6</sup>
	H <sub>2</sub>	Human recombinant	[ <sup>125</sup> I]APT	>1 × 10 <sup>-6</sup>
Muscarinic	M <sub>1</sub>	Human recombinant	[ <sup>3</sup> H]Pirenzepine	>1 × 10 <sup>-6</sup>
	M <sub>2</sub>	Human recombinant	[ <sup>3</sup> H]AF-DX384	>1 × 10 <sup>-6</sup>
	M <sub>3</sub>	Human recombinant	[ <sup>3</sup> H]4-DAMP	>1 × 10 <sup>-6</sup>
Neurokinine	NK <sub>2</sub>	Human recombinant	[ <sup>125</sup> I]NKA	>1 × 10 <sup>-6</sup>
	NK <sub>3</sub>	Human recombinant	[ <sup>3</sup> H]SR142801	>1 × 10 <sup>-6</sup>
Neuropeptide	Y <sub>1</sub>	SK-N-MC cells	[ <sup>125</sup> I]Peptide YY	>1 × 10 <sup>-6</sup>
	Y <sub>2</sub>	KAN-TS cells	[ <sup>125</sup> I]Peptide YY	>1 × 10 <sup>-6</sup>
Opioid	δ <sub>1</sub>	Human recombinant	[ <sup>3</sup> H]DADLE	>1 × 10 <sup>-6</sup>
	κ	Guinea-pig cerebellum	[ <sup>3</sup> H]U69593	>1 × 10 <sup>-6</sup>
	μ	Human recombinant	[ <sup>3</sup> H]DAMGO	>1 × 10 <sup>-6</sup>
Serotonin	5-HT <sub>1A</sub>	Human recombinant	8-OH-[ <sup>3</sup> H]DPAT	1.368 × 10 <sup>-8</sup>
	5-HT <sub>1B</sub>	Rat cerebral cortex	[ <sup>125</sup> I]Cytochrome P-450 + 30 μM propranolol	>1 × 10 <sup>-6</sup>
	5-HT <sub>2</sub>	Human recombinant	[ <sup>3</sup> H]BRL-43694	8.929 × 10 <sup>-6</sup>
	5-HT <sub>2A</sub>	Human recombinant	[ <sup>3</sup> H]LSD	>1 × 10 <sup>-6</sup>
	5-HT <sub>2B</sub>	Human recombinant	[ <sup>3</sup> H]LSD	>1 × 10 <sup>-6</sup>
	5-HT <sub>7</sub>	Human recombinant	[ <sup>3</sup> H]LSD	>1 × 10 <sup>-6</sup>
Norepinephrine transporter		Human recombinant	[ <sup>3</sup> H]Nisoxetine	>1 × 10 <sup>-6</sup>
Dopamine transporter		Human recombinant	[ <sup>3</sup> H]BTCF	>1 × 10 <sup>-6</sup>

DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CGS21680, 2-[p-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamidoadenosine; AB-MECA, 4-aminobenzyl-5'-N-methylcarboxamidoadenosine; RX821002, (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazolyl)-2-imidazole; CGP12177, 4-[3-[(1,1-dimethyl-2-hydroxypropyl)-1,3-dihydro-2H-benzimidazol-2-yl]-2-pyridyl]-1,4-benzodioxan-2-yl)-2-imidazole; SCH23390, R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; APT, aminopotentidine; AFDX384, (±)-5,11-dihydro-11-[(2-[2-(dipropylamino)methyl]-1-piperidinyl)ethyl]amino)carbonyl-6H-pyrido[2,3-b](1,4)benzodiazepine-6-one; 4-DAMP, 4-diphenylacetoxy-N-methylpiperazine methiodide; NKA, neurokinin A; SR142801, S-(N)-1-[3-[1-benzoyl-2-(3,4-dichlorophenyl)piperidin-3-yl]propyl]-4-phenylpiperidin-4-yl-N-methylacetamide; DADLE, [D-Ala<sup>2</sup>, D-Leu<sup>5</sup>]-enkephalin; U69593, (+)-[S-(+)-8β]-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide; DAMGO, [D-Ala<sup>2</sup>, N-Me-Phe<sup>4</sup>, Gly<sup>5</sup>-ol]-enkephalin; LSD, lysergic acid diethylamide; BTCF, N-[1-(2-benz(b)thiophenyl) cyclohexyl] piperidine.

dose of 1.25 g/kg i.p., and then polyethylene cannulas were inserted into the right common carotid artery and vein to measure the blood pressure and to administer 5-HT, respectively. The duodenum was incised, decorticated 2 to 3 cm from the stomach and cannulated for intraduodenal administration of the test drugs. Blood pressure was monitored using a pressure amplifier (AP-601G; Nihon Kohden), and the mean heart rate was recorded by a tachometer (AT-601G; Nihon Kohden) triggered by blood pressure pulsation. 5-HT was injected i.v. at 300 μg/kg to evoke a transient bradycardia [von Bezold-Jarisch (BJ) reflex]. After recovery to normal blood pressure and heart rate, the test drugs and vehicle (0.5% Tween 80) were administered into the duodenum. Thirty minutes later, 5-HT was readministered i.v., and the bradycardia was assessed.

**Wrap Restraint Stress-Induced Defecation in Rats.** The method described by Williams et al. (1988) was used with slight modifications. Rats were acclimated to observation metal mesh cages placed on a tray from the day before the experiment. After acclimation, the rats were lightly anesthetized with ether, and their fore-shoulder, upper forelimbs, and thoracic trunks were wrapped with adhesive tape (2.5 × 50 cm). Immediately after wrapping, the rats were returned to the observation cages. The number of feces was counted at 1 h after the wrapping. Test drugs and vehicle (0.5% Tween 80) were administered p.o. 1 h before the restraint. In the antagonism assays, TZB-30878 or vehicle (saline containing diluted hydrochloric acid) was injected i.p. 20 min after s.c. treatment with the vehicle (saline) or a selective 5-HT<sub>1A</sub> receptor antagonist, WAY-100635. Five minutes later, the animals were wrapped for 1 h, and the number of feces was counted.

**Data Analysis and Statistical Evaluation.** GraphPad Prism (version 4.00; GraphPad Software Inc., San Diego, CA) was used for data analyses in all assays. Specific binding of the radiolabeled ligand was calculated and a saturation binding curve was generated by plotting  $F (= X)$ , unbound radiolabeled ligand and  $B (= Y)$ , bound radiolabeled ligand on the horizontal and vertical axes, respectively, to calculate  $K_d$  values using nonlinear regression. The equation (one-site binding) was as follows:  $Y = B_{max} \times X / (K_d + X)$ .

A substitution curve was generated by plotting the test drug concentrations and binding inhibition rates on the horizontal and vertical axes, respectively, to calculate the concentration that corresponded to a 50% binding inhibition rate (IC<sub>50</sub>). The binding inhibition constant ( $K_i$  value) of the test drugs was calculated according to the following equation:  $K_i = IC_{50} / (1 + [L]/K_d)$ , where  $L$  is the concentration of radiolabeled ligand added.

Specific binding of [<sup>35</sup>S]GTPγS was calculated; the results were then presented as percent increases in binding from the basal value, and dose-response curves were plotted and analyzed. The maximal stimulation ( $E_{max}$ ) achieved for each drug was expressed as percent the maximal response of R-(+)-8-OH-DPAT.

Statistical analysis was performed using SAS (ProcClinical Package version 6.0; SAS Institute, Tokyo, Japan). The dose response for the relative potency of the drugs was analyzed by analysis of variance followed by a nonparametric or parametric Dunnett multiple comparison test with the limit of significance level at  $P < 0.05$ . Results between the two groups were compared with the Wilcoxon test.

## Results

## In Vitro Properties

**Radioligand Binding Assays.** The binding assays of [ $^3$ H]TZB-30878 to human 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors were performed three times each. A stable saturation curve (Fig. 1) was obtained from the assays, which indicates that TZB-30878 is a compound that possesses binding affinity to human 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors. The  $K_d$  values of TZB-30878 toward 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors were  $6.771 \times 10^{-10} \pm 0.3292 \times 10^{-10}$  and  $8.903 \times 10^{-9} \pm 1.728 \times 10^{-9}$  M, respectively.

Next, saturation binding analyses were conducted three times for each of the ligands, [ $^3$ H]8-OH-DPAT, and [ $^3$ H]BRL-43694. By using 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors, the  $K_i$  values for TZB-30878 were compared with those for the 5-HT<sub>1A</sub> receptor agonist, tandospirone, and the 5-HT<sub>3</sub> receptor antagonist, alosetron. TZB-30878 inhibited the binding of 8-OH-DPAT and BRL-43694 to these receptors in a concentration-dependent manner (Fig. 2). These results and the previously described results for the binding affinity indicated that TZB-30878 binds specifically to both human 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors. Tandospirone and alosetron also inhibited binding of each radioligand to the receptors in a concentration-dependent manner. At the receptor binding level, the  $K_i$  for TZB-30878 was approximately 10 times greater than that for tandospirone and approximately  $\frac{1}{10}$  of that for alosetron.

The receptor selectivity of TZB-30878 for the 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors was 300 times or more higher than that for the other receptors or transporters (adenosines A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub>; adrenalinines  $\alpha_1$ ,  $\alpha_2$ , and  $\beta_1$ ; dopamines D<sub>1</sub> and D<sub>2</sub>; GABA; histamines H<sub>1</sub> and H<sub>2</sub>; muscarinics M<sub>1</sub>, M<sub>2</sub>, and M<sub>3</sub>; neuroleptins NK<sub>2</sub> and NK<sub>3</sub>; neuropeptides Y<sub>1</sub> and Y<sub>2</sub>; opioids  $\delta$ ,  $\kappa$ , and  $\mu$ ; serotoninins 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, and 5-HT<sub>7</sub>; norepinephrine transporter and dopamine transporter) (Table 1).

**5-HT<sub>1A</sub> Agonist-Induced [ $^{35}$ S]GTP $\gamma$ S Binding Assays.** The functional activity of TZB-30878 on the 5-HT<sub>1A</sub> receptor was compared with that of other 5-HT<sub>1A</sub> receptor agonists and antagonist (Fig. 3; Table 2). A 5-HT<sub>1A</sub> receptor agonist, R-(+)-8-OH-DPAT, increased binding of [ $^{35}$ S]GTP $\gamma$ S to the receptor in a concentration-dependent manner. Generally, R-(+)-8-OH-DPAT is known to be a full agonist, and we have preliminarily confirmed that R-(+)-8-OH-DPAT and 5-HT exhibited comparable  $E_{max}$  values. S-(-)-8-OH-DPAT and tandospirone also increased binding of [ $^{35}$ S]GTP $\gamma$ S in a concentration-dependent man-

TABLE 2

Ligand properties of [ $^{35}$ S]GTP $\gamma$ S binding in human 5-HT<sub>1A</sub> receptors. Results are expressed as a percentage of the maximal response of R-(+)-8-OH-DPAT.

Drug	$E_{max}$ %
R-(+)-8-OH-DPAT	100
S-(-)-8-OH-DPAT	81.3
Tandospirone	66.7
TZB-30878	99.2
WAY-100635	7.2

ner.  $E_{max}$  values were 81.3% for S-(-)-8-OH-DPAT and 66.7% for tandospirone compared with that for R-(+)-8-OH-DPAT. This result indicated that S-(-)-8-OH-DPAT and tandospirone were partial agonists. The 5-HT<sub>1A</sub> receptor antagonist, WAY-100635, did not affect the binding of [ $^{35}$ S]GTP $\gamma$ S. TZB-30878 increased the binding of [ $^{35}$ S]GTP $\gamma$ S in a concentration-dependent manner, and the  $E_{max}$  value was comparable with that of R-(+)-8-OH-DPAT.

**Inhibition of 5-HT<sub>3</sub> Receptor-Mediated Contractions in Guinea Pig Ileum.** The functional activity of TZB-30878 on the 5-HT<sub>3</sub> receptor was compared with that of alosetron (Fig. 4). TZB-30878 alone did not affect longitudinal muscle strips with the myenteric plexus (data not shown). TZB-30878 suppressed the contractions caused by the 5-HT<sub>3</sub> receptor agonist, 2-methyl-5-HT, in a concentration-dependent manner and nearly fully suppressed the contractions at 1  $\mu$ M. This indicated that TZB-30878 possesses functional activity as a 5-HT<sub>3</sub> receptor antagonist. In addition, the 50% suppression concentration (IC<sub>50</sub>) of TZB-30878 was 0.014  $\mu$ M. Alosetron showed similar contraction-suppressive effects with an IC<sub>50</sub> of 0.017  $\mu$ M.

## In Vivo Properties

**5-HT<sub>1A</sub> Receptor-Mediated Behavior and Hypothermia in Rats.** TZB-30878 and tandospirone induced increases in flat body posture and lower lip retraction scores in rats (Figs. 5, A' and B). TZB-30878 produced a dose-related effect on flat body posture and lower lip retraction scores. TZB-30878 and tandospirone induced statistically significant behavioral changes at 10 mg/kg or at 3 mg/kg or higher, respectively. In addition, TZB-30878 and tandospirone also decreased rectal temperature (Fig. 5C), which attained statistical significance at 3 mg/kg or higher. It was clarified that TZB-30878 stimulates the 5-HT<sub>1A</sub> receptor in vivo.

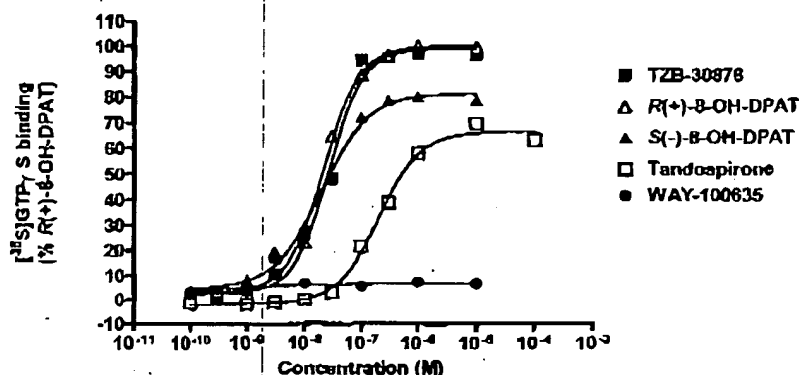


Fig. 3. The effects of several 5-HT<sub>1A</sub> ligands on [ $^{35}$ S]GTP $\gamma$ S binding to human 5-HT<sub>1A</sub> receptors expressed in CHO cells. (Specific [ $^{35}$ S]GTP $\gamma$ S binding was measured in the presence of various concentrations of the test drug. Non-specific binding was detected in the presence of 10  $\mu$ M GTP $\gamma$ S. Results are expressed as a percentage of the maximal response of R-(+)-8-OH-DPAT.)

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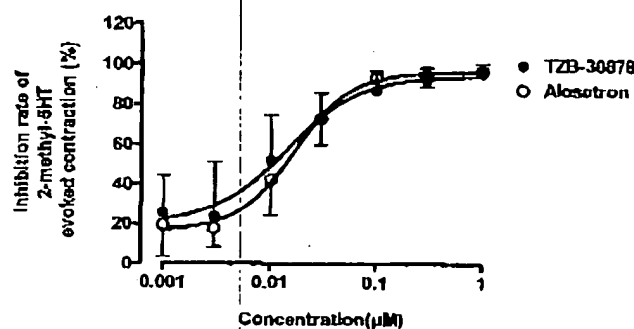


Fig. 4. The effects of TZB-30878 and alosetron on 2-methyl-5-HT-evoked contractions in guinea pig ileum longitudinal muscle strips including the myenteric plexus. Inhibition rates are represented as a percentage of 10  $\mu$ M 2-methyl-5-HT evoked contraction.  $n = 5-6$ /group. Values represent means  $\pm$  S.D.; 50% inhibition concentration was calculated by the dose-response curve.

**5-HT-Induced Bradycardia (BJ Reflex) in Rats.** Intraduodenal administration of TZB-30878 (0.01–3 mg/kg) or alosetron (0.001–0.3 mg/kg) inhibited bradycardia induced by 5-HT (Fig. 6) in a dose-dependent manner. The 50% inhibitory doses were 0.152 and 0.00678 mg/kg, respectively. The BJ reflex is known to be mediated by 5-HT<sub>3</sub> receptors. The maximum inhibitory action of both drugs reached the vehicle level, which clearly indicated that TZB-30878 possesses 5-HT<sub>3</sub> receptor antagonistic action in vivo.

#### Animal Model

**Wrap Restraint Stress-Induced Defecation in Rats.** Wrap restraint stress caused a significant increase in defecation (Fig. 7). Stress-induced defecation was reduced by prior administration of TZB-30878 in a dose-dependent manner. The dose of 1 mg/kg exhibited no effects whereas both the 3 and 10 mg/kg doses significantly reduced stress-induced defecation. In addition, TZB-30878 strongly suppressed stress-induced defecation and completely normalized the number of defecations. These results indicated the effectiveness of oral administration of TZB-30878 for stress-induced defecation. Prior administration of alosetron (3 and 10 mg/kg) also significantly reduced stress-induced defecation. However, alosetron did not normalize the number of defecations, and the maximum effect plateaued. On the other hand, tansospirone did not suppress stress-induced defecation at doses up to 100 mg/kg. The data on individual defecations showed a mixture of both suppressive and increasing trends.

To clarify the contribution of 5-HT<sub>1A</sub> receptors to the reduction of stress-induced defecation by TZB-30878, the effect of WAY-100635, a 5-HT<sub>1A</sub> receptor antagonist, was investigated (Fig. 8). After i.p. administration, TZB-30878 suppressed stress-induced defecation in a dose-dependent manner, which became statistically significant at 1 and 3 mg/kg. When WAY-100635 was administered before administration of TZB-30878, the suppressive effect of TZB-30878 was significantly reduced. However, the antagonistic effect of WAY-100635 did not completely eliminate the suppressive effect of TZB-30878 on stress-induced defecation.

#### Discussion

IBS is associated with psychological stress and alterations of gut motor function. We synthesized a novel compound, TZB-30878, which has dual pharmacological activities: it is

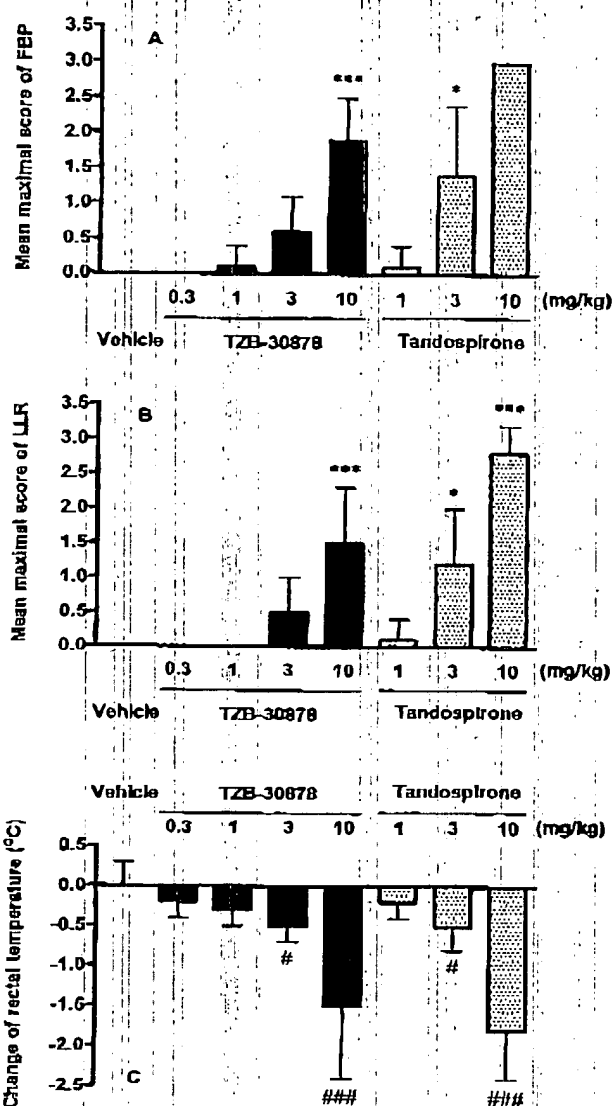


Fig. 5. The effects of TZB-30878 and tansospirone on 5-HT<sub>1A</sub> receptor-mediated behavior and rectal temperature in rats. Vehicle or drugs were injected i.p. and behavioral responses [flat body posture (FBP) and lower lip retraction (LLR)] were measured sequentially using a 0 to 3 scale. Maximal scores for FBP (A) and LLR (B) were adopted. Before and after administration, rectal temperature was measured (C).  $n = 10$ /group. Values represent means  $\pm$  S.D. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$  versus vehicle, nonparametric Dunnett multiple comparison test. #,  $P < 0.05$ ; ###,  $P < 0.001$  versus vehicle, parametric Dunnett multiple comparison test.

expected to have an antistress effect via 5-HT<sub>1A</sub> agonism and an antihyperdefecation effect via 5-HT<sub>3</sub> antagonism. The purpose of this study was to determine the effects of TZB-30878 in an IBS-like animal model. The present assays provide initial information that shows the importance and the usefulness of having both actions for the treatment of IBS.

The binding assays demonstrated that [<sup>3</sup>H]TZB-30878 bound to human 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors with a high affinity in a reversible and saturable manner. In addition, a competitive analysis revealed that TZB-30878 selectively inhibited binding of well known ligands to human 5-HT<sub>1A</sub> and 5-HT<sub>3</sub> receptors, as well as to specific ligands (tansospirone and alosetron). The binding inhibition constant ( $K_i$  value) of

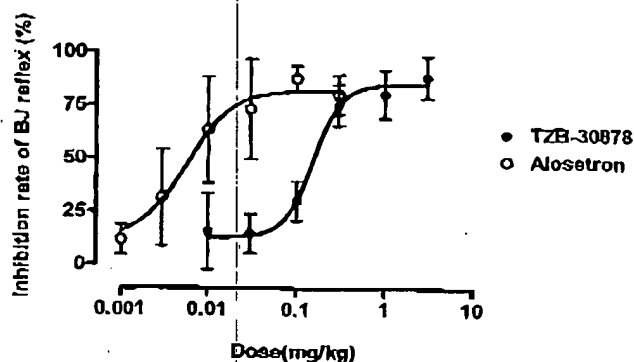


Fig. 6. The effects of TZB-30878 and alosetron on 5-HT-induced bradycardia in rats. Anesthetized rats were injected i.v. with 5-HT (300  $\mu$ g/kg) to evoke a transient bradycardia. After recovery, vehicle or test drugs were injected intraduodenally, and 5-HT was readministered 30 min later.  $n = 6$ /group. Values represent mean ( $\pm$ S.D.).

TZB-30878 was 10 times greater than that of tandospirone and  $1/10$  that of alosetron. Furthermore, the results of the competitive analysis of the rat hippocampus (5-HT<sub>1A</sub>) and entorhinal cortex (5-HT<sub>2</sub>) showed similar affinities of TZB-30878 to human and rat receptors (data not shown). In addition, as TZB-30878 did not show affinity to any other typical receptors, its selectivity to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors was confirmed.

The relative efficacy of 5-HT<sub>1A</sub> receptor agonists can be

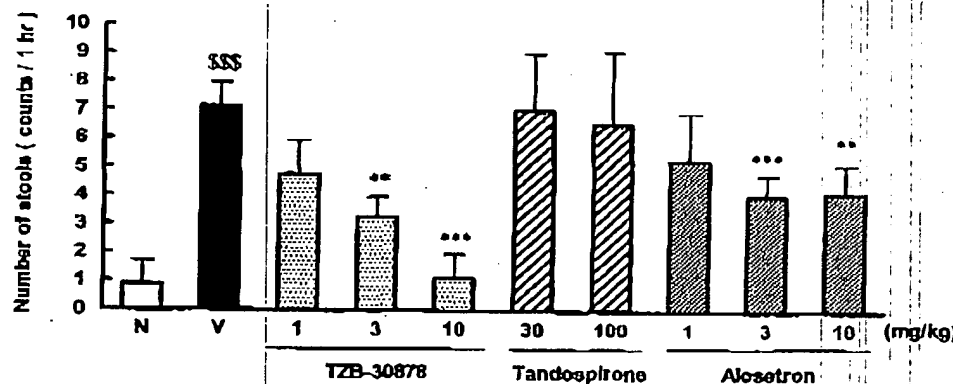


Fig. 7. The effects of TZB-30878, tandospirone, and alosetron on stress-induced defecation in rats. The upper bodies of lightly anesthetized rats were wrapped with adhesive tape, and the animals were returned to the observation cages. The number of feces dropped on the tray was counted 1 h after the wrapping. Vehicle or test drugs were administered p.o. 1 h before the restraint.  $n = 8$ /group. Values represent means  $\pm$  S.D. \$\$\$,  $P < 0.001$  versus normal (N), Wilcoxon test. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  versus vehicle (V), nonparametric Dunn's multiple comparison test. Normal group represents animals without wrapping.

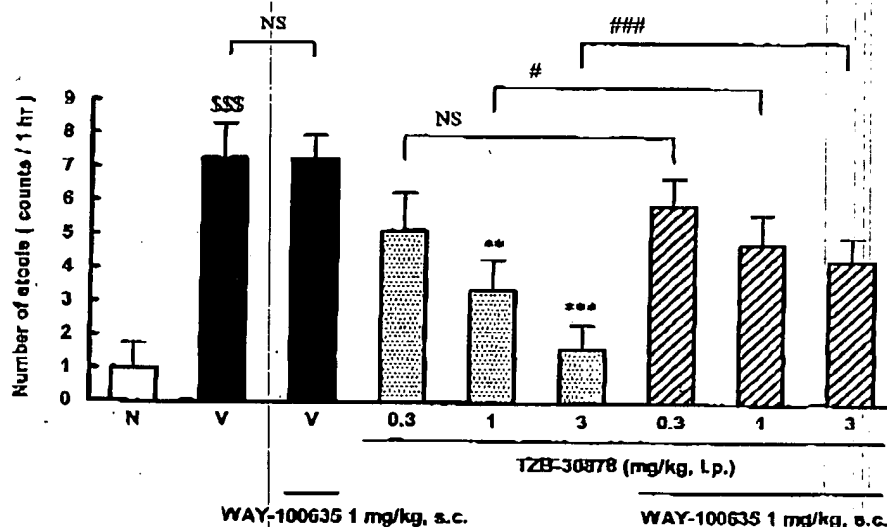


Fig. 8. The influence of 5-HT<sub>1A</sub> antagonist WAY-100635 on the inhibitory effect of TZB-30878 in stress-induced defecation. Vehicle or TZB-30878 was injected i.p. 20 min after s.c. injection with saline (containing diluted hydrochloric acid) or WAY-100635. Five minutes later, lightly anesthetized rats were wrapped for 1 h, and the number of feces was counted.  $n = 8$ /group. Values represent means  $\pm$  S.D. \$\$\$,  $P < 0.001$  versus normal (N), Wilcoxon test. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$  versus vehicle (V), nonparametric Dunn's multiple comparison test. #,  $P < 0.05$ ; ###,  $P < 0.001$  between indicated two groups, Wilcoxon test. NS, means nonsignificant. Normal group represents animals without wrapping.



tration of TZB-30878 induced a 5-HT syndrome and hypothermia, as with tandospirone. These results suggest that TZB-30878 stimulates 5-HT<sub>1A</sub> receptors in vivo, especially in the CNS.

The transient bradycardia induced by i.v. administration of 5-HT (von Bezold-Jarisch reflex) is the consequence of a reflex response of the vagus nerve after activation of sensory afferent fibers located mainly in the right ventricle (Paintal, 1973). The bradycardia induced by 5-HT was successfully inhibited by 5-HT<sub>3</sub> receptor antagonists (Fozard and Host, 1982). As a result, this inhibition is regarded as a useful index to assess in vivo 5-HT<sub>3</sub> receptor blocking activity. In the present experiments, TZB-30878 and alosetron inhibited the BJ reflex in a dose-dependent manner, which suggests that TZB-30878 is a 5-HT<sub>3</sub> antagonist that acts in vivo.

Williams et al. (1988) reported that wrap restraint stress caused intestinal signs in rats similar to IBS in humans without the formation of ulcers. The efficacy of anxiolytic agents, such as diazepam, and ant motility agents, such as trimebutine, for IBS has been evaluated in this model (Yamamoto et al., 1998). Thus, the wrap restraint-induced stress model is useful for assessing the effects of therapeutic agents for IBS. It has been reported that immobilization stress results in an increase in plasma 5-HT concentrations in rats, which indicates the possibility that 5-HT acts as a mediator of gut dysfunction caused by stress (Sharma and Dey, 1981). Most of the endogenous 5-HT is considered to be contained within the gastrointestinal tract, particularly within the enterochromaffin cells of the gut mucosa, and 5-HT-containing neurons have been identified in the enteric nervous system of several species (Costa et al., 1982). Endogenous 5-HT may mediate restraint stress-induced acceleration in bowel function through the 5-HT<sub>3</sub> receptor (Miyata et al., 1992). In the present study, TZB-30878 reduced restraint stress-induced defecation as did alosetron. It is thought that TZB-30878 reduced the defecation at least partially through 5-HT<sub>3</sub> antagonism at the doses used in this assay. It is interesting that, although alosetron only partially inhibited restraint stress-induced defecation, TZB-30878 completely normalized this sign. Moreover, the effect of TZB-30878 was partly inhibited by a 5-HT<sub>1A</sub> antagonist, WAY-100635. These results indicate that stimulation of the 5-HT<sub>1A</sub> receptors by TZB-30878 reduces restraint stress-induced defecation. Taken together, these findings indicate that the suppression of restraint stress-induced defecation by TZB-30878 was the result of the contribution of both 5-HT<sub>1A</sub> agonism and 5-HT<sub>3</sub> antagonism.

Miyata et al. (1992) reported that diazepam reduced restraint stress-induced defecation and suggested a potential anxiolytic action in this model. 5-HT<sub>1A</sub> receptor agonists have been reported to show an anxiolytic effect similar to that of benzodiazepines (Traber and Glaser, 1987; Gordon and Hen, 2004). Because TZB-30878 produced the 5-HT syndrome and permeated the blood-brain barrier, an anxiolytic effect via the CNS may contribute in part to the action of TZB-30878 in this IBS-like model. Furthermore, it has been reported that 5-HT<sub>3</sub> receptor antagonists also have an anxiolytic effect in animal models (Filip, 1992; Roychoudhury and Kulkarni, 1997). If TZB-30878 possesses an anxiolytic effect, we should consider it in relation to the effects of 5-HT<sub>3</sub> receptors on the CNS.

On the other hand, because 5-HT<sub>1A</sub> receptors are localized

in the enteric nerves (Galligan et al., 1988; Galligan, 1992), the 5-HT<sub>1A</sub> receptors in the myenteric plexus are likely to contribute to the reduction of the restraint stress-induced defecation. It has been reported that 5-HT<sub>1A</sub> receptor stimulation of the enteric neurons inhibits excitatory postsynaptic potentials and acetylcholine release in the myenteric plexus and smooth muscle contraction (Dietrich and Kilbinger, 1996). If 5-HT<sub>1A</sub> receptors negatively modulate 5-HT neuronal activity in the myenteric plexus, the effects of endogenous 5-HT through 5-HT<sub>3</sub> receptor stimulation may be reduced by TZB-30878. Further studies are needed to assess effects on the peripheral 5-HT<sub>1A</sub> receptors.

In contrast with TZB-30878, oral administration of tandospirone did not decrease restraint stress-induced defecation, although it induced the 5-HT syndrome via intraperitoneal administration. We have already confirmed that intraperitoneal administration of tandospirone partially and significantly reduced restraint stress-induced defecation by approximately 40% (unpublished observations). The inconsistency with the results for tandospirone may be due to its major metabolite, 1-(2-pyrimidinyl) piperazine (1-PP). After oral administration, tandospirone is rapidly metabolized to 1-PP, and 1-PP has low affinity to the 5-HT<sub>1A</sub> receptors but has high affinity to the  $\alpha_2$ -adrenoceptors (Miller et al., 1992). Because TZB-30878 does not possess the 1-PP structure, the effects of TZB-30878 are unrelated to 1-PP. It has been reported that 1-PP and other  $\alpha_2$ -adrenoceptor antagonists increased fecal excretion in rats (Crocchi and Bianchetti, 1992). Such effects of 1-PP in increasing fecal excretion may counteract the effects of 5-HT<sub>1A</sub> receptor agonists in reducing restraint stress-induced defecation. Therefore, we have to consider the oral bioavailability when evaluating the action of tandospirone.

The most important finding in this study was that the inhibitory effects of TZB-30878 on restraint stress-induced defecation may be mediated not only by 5-HT<sub>3</sub> receptor antagonism but also by 5-HT<sub>1A</sub> receptor agonism. For 5-HT<sub>3</sub> receptor antagonism, the activity of TZB-30878 was weak compared with that of alosetron both in vitro and in vivo, despite the fact that TZB-30878 had more powerful efficacy than alosetron in the IBS-like model. From another point of view, the 5-HT<sub>3</sub> receptor works concomitantly with 5-HT<sub>1A</sub> receptor agonism in this model. A new concept suggested by our results is that the dual actions, 5-HT<sub>1A</sub> agonism and 5-HT<sub>3</sub> antagonism, can be expected to have a benefit for the treatment of IBS, and TZB-30878 is the first compound to fit this concept. Currently, other 5-HT<sub>3</sub> receptor antagonists (i.e., cilansetron and ramosetron) for the treatment of d-IBS are under development. TZB-30878 could achieve an efficacy level higher than that for the existing 5-HT<sub>3</sub> receptor antagonists and is expected to be a next-generation therapeutic agent for d-IBS.

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